IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants: Linda G. Cima, Edward W. Merrill, and Philip R. Kuhl

Serial No.: 08/398,555 Group Art Unit: 1811

Filed: March 3, 1995 Examiner: Jeffrey E. Russel

For: *CELL GROWTH SUBSTRATES WITH TETHERED CELL GROWTH EFFECTOR MOLECULES*

Assistant Commissioner of Patents
Washington, D.C. 20231

APPEAL BRIEF

Sir:

This is an appeal from the final rejection of claims 1-32 in the Office Action mailed April 14, 1997 and maintained in the Advisory Actions mailed August 15, 1997 and September 16, 1997 in the above-identified patent application. A Notice of Appeal was mailed on September 24, 1997. A check in the amount of $155.00 for the filing of this Appellants' Brief is enclosed.

(1) REAL PARTIES IN INTEREST

The real parties in interest of this application are Massachusetts Institute of Technology, Cambridge, Massachusetts, the assignee, and Corning, Inc., Corning, New York, which has rights in the subject matter of the application.

(2) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to Appellants, the undersigned, or Appellants' assignee which directly affect, which would be directly affected by, or which would have a bearing on the Board's decision in this appeal.

(3) STATUS OF CLAIMS ON APPEAL

Claims 1-32 are pending and are on appeal. The text of each claim on appeal, as amended in the Amendment mailed January 23, 1997, is set forth in Appendix I to this Appeal Brief. Appellants have petitioned that the Amendment of September 4, 1997 be entered. Claims 1-6, 8-22, and 24-32 would be pending upon entry of the Amendment. The text of each claim on appeal, as would be amended in the Amendment mailed September 4, 1997, is set forth in Appendix II to this Appeal Brief. Also enclosed is a copy of the Amendment Under 37 C.F.R. §1.116 that was hand delivered September 4, 1997, and the Petition For Entry Of Amendment that was mailed September 24, 1997.

(4) STATUS OF AMENDMENTS

An Amendment to claims 1, 5, 6, 9, 13, 25, 29, 31, and 32, mailed January 23, 1997, was entered. Claims 1-32 are pending as amended. An Amendment mailed July 14, 1997 and an Amendment hand delivered September 4, 1997 were not entered. A Petition For Entry of the Amendment of September 4, 1997 was mailed September 24, 1997 in

response to the Advisory Actions mailed August 15, 1997 and September 16, 1997. Claims 1-6, 8-22, and 24-32 would be pending upon entry of the Amendment. The Petition has not been decided as of this date.

(5) SUMMARY OF THE INVENTION

Claims 1-31 are directed to compositions and methods for the growth of eukaryotic cells wherein the cell growth is enhanced through the use of "tethered growth factors". Claim 32 is directed to a method for testing the effect of a compound on tissue cells including exposing the compound to cells growing on the composition as defined in claim 1.

The compositions include a substrate having tethered thereto an effective concentration of one or more growth effector molecules to enhance the rate of target cell growth over the rate of target cell growth with soluble growth effector molecules (page 15, line 27- page 16, lines 1-2) and growth effector molecules merely adsorbed to a substrate, (page 24, lines 16-22) without internalization of the molecules (page 5, lines 23-30). The tethers are made from biocompatible, synthetic, water soluble polymers (page 6, line 23- page 7, line 2). The tethers are preferably branched so as to be able to covalently link more than one growth effector molecule (page 4, lines 16-18 and page 7, lines 3-20). The tether polymers may be polyethylene oxide, carboxymethylcellulose or starch (Page 6, line 27- page 7, line 2). The tether may have a backbone length between 5 and 50,000 atoms, between

100 and 50,000 atoms, or between 5 and 500 atoms (page 7, lines 10-20 and page 8, lines 8-12).

The substrate may have the form of netting, fibers, sponge or shaped polymers (page 9, lines 25-26). The shaped polymer may have the form of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape (page 9, lines 25-26). The substrate may be glass, metal, or biocompatible polymer (page 9, lines 9-24). The substrate polymer is synthetic or natural polymer (page 9, lines 9-24), such as a protein, polysaccharide, extracellular matrix protein, polyester, polycapralactone, polyhydroxybutyrate, polyanhydride, polyphosphazene, polyorthoester, polyurethane, or combination thereof (page 9, lines 9-24).

Examples of the growth effector molecules are epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, or combinations thereof (page 10, line 20- page 11, line 30).

The method for growing cells includes bringing the cells into contact with a composition. The composition may be brought into contact with the cells by administering the composition to a patient by injection, infusion or implantation (page 18, lines 16-21). For example, the substrate may be shaped to match a desired tissue shape and implanted (page 18, lines 5-8).

The cells may be stem or parenchymal cells, such as hepatocytes (page 14, lines 15-27).

A method of using the disclosed compositions for testing the effect of a compound on tissue cells includes exposing the compounds to the compositions (page 18, lines 24-30).

(6) ISSUES ON APPEAL

The issues presented on appeal are

(1) whether claims 1-32 should be rejected under 35 U.S.C. §112, second paragraph for indefiniteness;

(2) whether claim 8 should be rejected under 35 U.S.C. §112, fourth paragraph for failing to further limit the subject matter of a previous claim;

(3) whether claims 1-9, 13, 18-25, and 31 should be rejected under 35 U.S.C. §102(b) as disclosed by U.S. Patent No. 5,512,424 to Clapper et al. ("Clapper");

(4) whether claims 10-12 and 26-28 should be rejected under 35 U.S.C. §103 as obvious over Clapper;

(5) whether claims 1-7, 9, 10, 13, 18-21, 23, 25, 26, and 29-31 should be rejected under 35 U.S.C. §102(b) as disclosed by European Patent Application 531733 ("EP '733");

(6) whether claims 10-12 should be rejected under 35 U.S.C. §103 as obvious over EP '733;

(7) whether claims 1-10, 12-26, 28, and 31 should be rejected under 35 U.S.C. §102(b) as disclosed by WO 89/05616 by Bio-Metric Systems, Inc. ("WO '616");

(8) whether claims 11 and 27 should be rejected under 35 U.S.C. §103 as obvious over WO '616;

(9) whether claims 1-9, 13-16, 18-25, and 31 should be rejected under 35 U.S.C. §103 as obvious over U.S. Patent No. 5,370,681 to Herweck et al. ("Herweck"), in combination with U.S. Patent No. 5,171,264 to Merrill ("Merrill '264");

(10) whether claims 10-12 and 26-28 should be rejected under 35 U.S.C. §103 as obvious over Herweck in view of Merrill '264 and further in view of Merrill, J. Biomater. Sci. Polymer. 5, 1-11 (1993) ("Merrill");

(11) whether claim 17 should be rejected under 35 U.S.C. §103 as obvious over Herweck in combination with Merrill '264 in combination with U.S. Patent No. 5,522,895 to Mikos et al. ("Mikos")

(12) whether claims 29 and 32 should be rejected under 35 U.S.C. §103 as obvious over Herweck in combination with Merrill '264 and U.S. Patent No. 5,032,508 to Naughton et al. ("Naughton"); and

(13) whether claims 29 and 30 should be rejected under 35 U.S.C. §103 as obvious over Herweck in combination with Merrill '264 and Tomomura et al. J. Cell. Physiol. 30:221-227 (1987) ("Tomomura").

(7) GROUPING OF CLAIMS

The claims do not stand or fall together. The claims should be divided into at least the following six groups, each of which stands or falls independently of the other groups: i) claims 1-8, 13, 18-25, and 31; ii) claim 9; iii) claims 10-12 and 26-28; iv) claims 14-17; v) claims 29 and 30; and vi) claim 32. The group i claims are directed to compositions and methods for enhancing cell growth that include a biocompatible substrate, polymeric tethers, and growth effector molecules, wherein the molecules are covalently linked to one end of a tether and another end of the tether is covalently linked to the substrate. The group ii claim is directed to the composition as defined in claim 1, wherein the growth effector molecules are more specifically defined. The group iii claims are directed to the compositions and methods as defined in claims 1 and 13, wherein the tether lengths are more specifically defined. The group iv claims are directed to methods for growing eukaryotic cells using the compositions as described in the group i claims, comprising administering the composition to a patient, such as by implantation. The group v claims are directed to using the method as described in claim 13 with stem or parenchymal cells, such as hepatocytes. The group vi claim is directed to using the compositions as described in the group i claims for testing a compound for its effect on tissue by contacting the compound with the composition. All of the groups require a separate analysis as to patentability since they contain different elements.

(8) ARGUMENTS

(a) The Claimed Invention

The claims are directed to compositions and methods for enhancing cell growth or for testing the effect of a compound on tissue that include a biocompatible substrate, polymeric tethers, and growth effector molecules, wherein the molecules are covalently linked to one end of a tether and another end of the tether is covalently linked to the substrate. The growth effector molecules can freely interact with the cell but are not internalized by the cell.

Most currently used cell and tissue growth compositions include soluble growth effector molecules, such as growth factors, either as an additive or as a component of complex growth media. The use of soluble growth effector molecules has certain drawbacks such as loss in responsiveness to the molecules. Cells have a complex, nonlinear response to the concentration of growth factor in their environment and extended exposure to high growth factor concentrations may cause cells to lose responsiveness to the factor. For example, EGF, a potent mitogen for a wide variety of cell types and arguably the best-characterized of the growth factors, is typically internalized by the cell when delivered in soluble form, and the cell often responds by down-regulating the number of EGF receptors. This down-regulation causes cells to lose responsiveness to EGF. Because the growth effector molecules of the claimed compositions and methods are tethered, they cannot be internalized by the cells. Therefore, down-regulation is avoided.

The effectiveness of the growth effector molecules on the rate of cell growth is maintained, and in fact enhanced, because the growth effector molecules are tethered on the ends of flexible, water soluble, tethers that provide mobility to the molecules sufficient for the molecules to contact receptors in the cell membrane, but without allowing internalization of the molecules. The polymers of the claimed compositions and methods are soluble in aqueous solution and will extend to their full length, providing a wide range of movement (flexibility) to the factors attached thereto. This is a very important aspect of Appellants' tethers. As discussed in the application at page 6, lines 6-8 and 11-26 and page 7, lines 21-30, the tether must be flexible to allow the growth effector molecule to contact the receptor on the cell surface and also to allow the growth effector molecule-receptor complex to move within the cell membrane. See, for example, the Specification at page 6, line 19, "Substantial mobility of a tethered growth factor is critical . . . ."

Not only will the tethers used in Appellants' claimed compositions and methods extend and provide mobility to the attached molecules but the tethers will not interact with the cell. This attribute also allows free movement of the molecules so that the molecules can contact the cell receptors and allow aggregation of growth effector molecule/receptor complexes on the cell membrane. See the Specification at page 5, line 24 through page 6, line 10.

The molecules are attached in a concentration effective to enhance the rate of growth of the target cells over the rate of cell growth with soluble or adsorbed growth effector

molecules. The amount and types of tethers must be properly balanced with the amounts of growth effector molecules to achieve enhanced growth rate. Because the tethers are water soluble, they are flexible and allow substantial mobility to the attached molecules. However, the tethers are also cell repellent and therefore could discourage cell growth. The use of branched tethers, in particular, avoids this potential problem by minimizing the amount of tether material, while achieving effective growth effector molecule concentration. Appellants have avoided sterically hindering contact of growth effector molecules with the receptors in the cell membrane. Appellants show how to enhance the rate of cell growth as compared to the rate of cell growth with soluble or adsorbed molecules by balancing use of polymeric water soluble tethers which do not bind to cells and the use of the proper amounts of tethered growth effector molecules.

Another problem with the use of soluble growth effector molecules, which is avoided by the claimed compositions and methods, is that growth effector molecules, when placed in a complex cellular environment, often end up stimulating the growth of competing cells which then overgrow the target cells. Researchers have attempted to solve this problem by targeting delivery of factors at a specific site, but this approach is not always successful because soluble growth factors can readily diffuse into the blood stream and away from the target site, exerting their effects elsewhere. This diffusion of growth factors is also a problem because it increases the amount of growth factor that must be used in order to have the desired local effect. Internalization of growth factors and loss of responsiveness to

growth factors is a particular problem for *in vivo* applications considering the amount of time cell growth must be stimulated to allow wound healing.

Another attempt to improve the longevity of growth effector molecule effects *in vivo* has been to incorporate the molecules in a slow release material, such as by adsorbing the molecules to a substrate. Such a scheme still requires large amounts of growth effector molecules and does not address the problem of competing cell growth due to diffusion of the molecules. The large amount of growth effector molecules needed for these cell and tissue growth methods is a particular problem because these molecules are difficult and expensive to prepare.

The tethers are preferably branched and water soluble so that each tether is able to covalently bind more than one growth effector molecule. By using a multi-functional flexible tether, Appellants can go to very low factor concentrations and still achieve receptor aggregation by virtue of having more than one factor on each tether. So, even though the tethers can be very far apart (i.e. the distance from the center of one tether to the center of the adjacent tether is more than twice the fully extended chain length of the tether), receptor-receptor interactions can still occur in the membrane after growth effector molecule binding because the molecules are locally clustered. If linear tethers, i.e. tethers with only one attachment site for a growth effector molecule and one attachment site to the substrate, are used, going to lower concentrations also increases the distance between factors and potentially inhibits the ability of receptor-molecule complexes to interact in the cell

membrane. Thus, at lower concentrations, signalling may not occur at all using linear tethers, because the molecules are homogeneously spaced on the surface.

Appellants' tethers can bind more than one molecule of the same growth effector or can bind different growth effector molecules. Thus, the density of a growth effector molecule on a substrate can be increased without substantially increasing the number of cell-repellant tethers. Alternatively, for example, both insulin and EGF could be tethered to the same substrate, allowing presentation of two or more molecules to the cell.

(b) Rejections Under 35 U.S.C. §112

Claims 1-32 were rejected under 35 U.S.C. §112, second paragraph, and claim 8 was rejected under 35 U.S.C. §112, fourth paragraph. In particular, the phrase "to enhance the rate of target cell growth" was objected to, for allegedly failing to provide a basis for comparison of cell growth so that it can be determined whether growth is "enhanced". It is also argued that the term "polymer" in claims 5, 6, 21, and 22 is indefinite because it is not clear which polymer is meant. As for claim 8, the term "starch" is objected to because it is not a synthetic polymer, as required by claim 1.

i. The Applicable Legal Standard For §112

The correct legal standard is that the claims must not be read in a vacuum but rather must be read in light of the supporting specification and the relevant prior art as they would

have been read by one skilled in the art. *In re Moore*, 439 F.2d 1232, 1235, 169 U.S.P.Q. 236, 238 (CCPA 1971).

ii. The Claims Are Definite

The §112 rejections should not be sustained on appeal because one skilled in the art can reasonably ascertain the scope of the claimed compositions and methods. The meaning of the term "enhance" is generally understood and further can be readily understood from the supporting specification. The purpose of the claimed methods and compositions, as can be readily discerned from even a cursory reading, is to enhance the rate of cell growth using substrates and growth effector molecules tethered to the substrates, as opposed to culturing the cells on a substrate in the presence of soluble molecules or molecules merely adsorbed to a substrate. See, for example, page 5, lines 6-30. Moreover, the term is specifically defined in the specification at, for example, page 15, line 27- page 16, lines 1-2, page 24, lines 16-22 and the Figures. Therefore, the claims are definite because one of skill in the art can ascertain their scope.

iii. The Claims Properly Limit The Preceding Claims

Claims 5 and 21 depend on claims 4 and 20, respectively. Claim 4 recites "wherein the biocompatible substrate is selected from the group consisting of glasses, metals, and biocompatible polymers." Claim 20 is similar except metals are not included. It is clear,

therefore, that the term polymer in claims 5 and 21 refers to the biocompatible polymer of the substrate that is referred to in claims 4 and 20. Claims 6 and 22 depend on claims 5 and 21, respectively, and thus refer to the same polymers. Therefore, these claims are also definite.

iv. The Claim Amendments

For clarity, independent claims 1, 13, 31, and 32 are proposed to be amended to recite that the rate of target cell growth is enhanced over the rate of cell growth with soluble or adsorbed growth effector molecules. Claims 5, 6, 21, and 22 are amended to clarify that it is the substrate polymer that is described in these claims. Claim 8 is amended to delete "starch" from the list of tether materials. In the Examiner's Interview Summary Record of the Interview conducted August 29, 1997 and in the Advisory Action of September 16, 1997, it was stated that these proposed amendments would overcome all of the §112 rejections.

(c) Rejections Under 35 U.S.C. §102(b)

Claims 1-9, 13, 18-25, and 31 were rejected under 35 U.S.C. §102(b) as disclosed by Clapper. Claims 1-7, 9, 10, 13, 18-21, 23, 25, 26, and 29-31 were rejected under 35 U.S.C. §102(b) as disclosed by EP '733. Claims 1-10, 12-26, 28, and 31 were rejected under 35 U.S.C. §102(b) as disclosed by WO '616.

i. The Applicable Legal Standard For §102

Anticipation requires the disclosure, in a single prior art reference, of every element of the claim. *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 90 (Fed. Cir. 1986). Absence of a claimed element from a prior art reference negates anticipation. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 224 U.S.P.Q. 409 (Fed. Cir. 1984). Appellants submit that the prior art references cited by the Examiner fail to disclose all the limitations of the claimed compositions and methods.

ii. Clapper Does Not Disclose Every Element Of The Claims

Clapper discloses a cell culture support which includes a positively charged molecule, which can be a polymer such as polylysine, and a cell adhesion factor, i.e. a factor which enhances cell adhesion, not cell growth. The cell adhesion factor and the positively charged molecule are covalently linked to the support and, in one embodiment, the cell adhesion factor is covalently bound to the positively charged molecule, either of which is attached to the support. The cell adhesion factor and the positively charged molecule are "used at a surface density sufficient to promote initial cell density and to stabilize attachment of the cells to the surface." Clapper at col. 7, lines 7-10, and at col. 4, lines 30-35.

Clapper does not disclose a composition for stimulating the growth of eukaryotic cells wherein the rate of cell growth is enhanced by presentation of an effective amount of growth factor tethered to the surface of a substrate. Clapper, in distinction, discloses that the

composition taught therein merely improves cell attachment and stabilizes cell growth. See Clapper abstract, and column 4, lines 63-67. *A priori*, stabilization is not enhancement.

In contrast, the claimed composition comprises tethered growth effector molecules "attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules." As discussed at pages 11 and 20-22 of the specification, the enhancement of cell growth was measured by DNA synthesis. Such measurements showed that the rate of cell growth was enhanced over cells grown with soluble growth effector molecule and cells grown with growth effector molecules merely adsorbed to the substrate (see the Examples and Figures 1 and 2). Clapper discusses measurements of cell attachment (Examples 3 and 6-10), and measurements of cell growth which showed only that cell growth was not decreased (column 20, lines 53-57 and column 22, lines 4-7). In other words, enhanced cell growth compared to cell growth in the presence of soluble or adsorbed growth effector molecule was not demonstrated.

Moreover, Clapper teaches away from the claimed compositions and methods because Clapper's use of positively charged molecules as tethers encourages cell adhesion to the tethers. Indeed, that is the goal! If Clapper's tethers were used in Appellants compositions and methods they would discourage the interaction of the growth effector molecules with the cell receptors. Appellants' use of flexible tethers that do not interact with the cell allows of

the factors to the receptors and allows aggregation of growth effector molecule/receptor complexes on the cell membrane. See the Specification at page 5, line 24 through page 6, line 10.

The claims as they are proposed to be amended even more clearly distinguish the compositions and methods over Clapper because Clapper does not disclose the use of branched tethers where each tether is able to covalently link more than one growth effector molecule.

iii. EP '733 Does Not Disclose Every Element Of The Claims

EP '733 discloses a carrier to which is immobilized a cell growth factor. The factor may be attached to the carrier through a linker or spacer that is preferably about 2 nm in length (page 3, line 53). The spacer may be a polymer such as polyethyleneimine, polyamino acid or polymethylene (page 3, lines 56-58). It is stated that the presence of a cationic group on the spacer molecule is desirable to aid in cell adsorption to the surface through electrostatic force (page 4, lines 1-3). EP '733 does not teach or suggest a growth effector molecule "tethered" to a substrate as claimed by Appellants but rather discloses a cell growth factor "immobilized" on a substrate (see claim 1 and page 2, lines 8 and 48). Thus EP '733 discloses a composition similar to the adsorbed cell growth factor compositions to which Appellants compare their tethered compositions and demonstrate that the two compositions are not equivalent. See Figure 2 and the discussion at page 24, where it is

demonstrated that Appellants' tethered growth effector molecules enhance cell growth as compared to adsorbed growth effector molecules.

Polyethyleneimine is positively charged and will attract and interact with proteins and cells, thus inhibiting free movement of the tethered molecules. On the other hand, the polymers of the claimed compositions and methods do not interact with cells but will provide a wide range of movement (flexibility) to the factors attached thereto. This is a very important aspect of Appellants' tethers. As discussed in the application at page 6, lines 6-8 and 11-26 and page 7, lines 21-30, the tether must be flexible to allow the growth effector molecule to contact the receptor on the cell surface and also to allow the growth effector molecule-receptor complex to move within the cell membrane. See, for example, the Specification at page 6, line 19, "Substantial mobility of a tethered growth factor is critical . . . .") EP '733 thus teaches away from the Appellants' compositions and methods, because it teaches use of polymers that will interact with the cell.

EP '733 does not disclose that the compositions taught therein enhance the rate of cell growth over the rate of cell growth due to soluble or adsorbed growth effector molecule. Data is given comparing the cell density after four days for a sample with immobilized insulin (Comparative Example 2) and a sample without immobilized insulin or immobilized cell adhesion factor RGD (Comparative Example 3), i. e. samples with and without insulin. Data is not given for cells grown with soluble insulin. Moreover, there is no recognition

that growth rates can be enhanced by tethering a specific concentration of growth factors on a substrate.

iv. WO '616 Does Not Disclose Every Element Of The Claims

WO '616 generally discloses the use of polymeric spacers to distance a biomolecule from a substrate. Among the biomolecules mentioned are some of the growth effector molecules claimed by Appellants. However, the only examples in WO '616 involving cells demonstrate tethering of collagen, hyaluronic acid, and fibronectin (Examples 3 and 6). WO '616 does not report enhanced growth of cells but only enhanced adhesion as recognized at page 24, "The cells preferentially attached to the modified surface versus control surfaces, as indicated by the distance they grew out over the plastic surface." Moreover, the results reported are not comparisons of tethered growth effector molecules versus soluble or adsorbed growth effector molecules.

WO '616 does not teach how to tether EGF to alter cell growth. WO '616 teaches the use of PEO (a polymer commonly grafted to surfaces to inhibit cell adhesion), and generally teaches high concentrations of tether with no specifics about how to cause cell adhesion in the presence of this type of tether so as to avoid rounding up and non-adherent cells. Appellants show how to enhance the rate of cell growth as compared to the rate of cell growth with soluble or adsorbed molecules by balancing use of polymeric water soluble tethers which do not bind to cells and the use of the proper amounts of tethered growth

effector molecules. WO '616 teaches how to tether proteins, but not how to tether EGF so that cell growth will be enhanced.

With respect in particular to the claims as they are proposed to be amended, WO '616 discloses only linear polymeric tethers having one end attached to a support and the second end attached to a biomolecule. In other words, WO '616 does not teach or suggest that the tethers can bind more than one biomolecule, as claimed by Appellants. This is an important aspect of the claimed compositions and methods, as discussed in the application on page 7, lines 3-8 and page 12, lines 25-28. Appellants' tethers can bind more than one molecule of the same growth effector or can bind different types of growth effector molecules. Thus, the density of a growth effector molecule on a substrate can be increased without substantially increasing the number of cell-repellant tethers. Alternatively, for example, both insulin and EGF could be tethered to the same substrate, allowing presentation of two or more molecules to the cell.

Under the approach outlined in WO '616, in theory any concentration of molecules could be attached. However, since only linear tethers, i.e. tethers with only one attachment site for a factor and one attachment site to the substrate, are used, going to lower concentrations also increases the distance between factors and potentially inhibits the ability of receptor-factor complexes to interact in the cell membrane. Thus, at lower concentrations, signalling may not occur at all using linear tethers, because the factors are homogeneously spaced on the surface. By using a multi-functional flexible tether, Appellants

can go to very low factor concentrations and still allow receptor aggregation by virtue of having more than one factor on each tether. Even though the tethers can be very far apart (i.e. the distance from the center of one tether to the center of the adjacent tether can be more than twice the fully extended chain length of the tether), receptor-receptor interactions can still occur in the membrane after ligand-binding because the factors are locally clustered.

(d) Rejections Under 35 U.S.C. §103

Claims 10-12 and 26-28 were rejected under 35 U.S.C. §103 as obvious over Clapper. Claims 10-12 were rejected under 35 U.S.C. §103 as obvious over EP '733. Claims 11 and 27 were rejected under 35 U.S.C. §103 as obvious over WO '616. Claims 1-9, 13-16, 18-25, and 31 were rejected under 35 U.S.C. §103 as obvious over Herweck in combination with Merrill '264. Claims 10-12 and 26-28 were rejected under 35 U.S.C. §103 as obvious over Herweck in view of Merrill '264 and further in view of Merrill. Claim 17 was rejected under 35 U.S.C. §103 as obvious over Herweck in combination with Merrill '264 in combination with Mikos. Claims 29 and 32 were rejected under 35 U.S.C. §103 as obvious over Herweck in combination with Merrill '264 and Naughton. Claims 29 and 30 were rejected under 35 U.S.C. §103 as obvious over Herweck in combination with Merrill '264 and Tomomura.

i. The Applicable Legal Standard For §103

The U.S. Patent and Trademark Office has the burden under 35 U.S.C. §103 to establish a *prima facie* case of obviousness. *In re Warner et al.*, 379 F.2d 1011, 154 U.S.P.Q. 173, 177 (C.C.P.A. 1967), *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598-99 (Fed. Cir. 1988). In rejecting a claim under 35 U.S.C. §103, the Examiner must establish a *prima facie* case that: (a) the prior art suggests the claimed invention; and (b) the prior art indicates that the invention would have a reasonable likelihood of success. *In re Dow Chemical Company*, 837 F.2d 469, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988). A *prima facie* case of obviousness cannot be established by hindsight reconstruction. The prior art must provide one of ordinary skill in the art with the motivation to make the proposed modifications needed to arrive at the claimed invention. *In re Geiger*, 815 F.2d 686, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987); *In re Lalu and Foulletier*, 747 F.2d 703, 705, 223 U.S.P.Q. 1257, 1258 (Fed. Cir. 1984). A claimed invention is not *prima facie* obvious if the primary references do not suggest all elements of the claimed invention and the prior art does not suggest the modifications that would bring the primary references into conformity with the claims at issue. *In re Fritch*, 23 U.S.P.Q.2d, 1780 (Fed. Cir. 1992). *In re Laskowski*, 871 F.2d 115 (Fed. Cir. 1989). This is not possible when the claimed invention achieves more than what any or all of the prior art references allegedly suggest, expressly or by reasonable implication. Further, a reference which leads one of ordinary skill in the art

away from the claimed invention cannot render it unpatentably obvious. *Dow Chem. Co. v. American Cyanamid Co.* 816 F.2d 617, 2 USPQ2d 1350 (Fed. Cir. 1987).

Moreover, there must be some motivation to combine the references. "There must be some reason, suggestion, or motivation found in the prior art whereby a person of ordinary skill in the field of the invention would make the combination." *In re Oetiker*, 24 USPQ2d 1443 (Fed. Cir. 1992).

ii. Clapper Does Not Render The Claims Obvious

As discussed above in Section (b)i, Clapper does not disclose a composition or method for stimulating cell growth wherein the rate of target cell growth is enhanced. Nor does Clapper suggest attaching growth effector molecules to a tether attached to a substrate at a concentration effective to enhance the rate of cell growth. Clapper does not suggest how this might be accomplished using the positively charged molecules to which cell adhesion is encouraged. In the claimed compositions and methods, flexible tethers allow the growth effector molecules access to cell receptors and allow aggregation of receptor/growth effector molecule complexes in the membrane. The Examiner's argument is that one of skill in the art would have been motivated to determine all operable and optimal backbone lengths for the positively charged molecules of Clapper. However, since Clapper does not render obvious the underlying independent claims, it also does not render obvious the claims dependent thereon, which specifically define tether length (claims 10-12 and 26-28).

Moreover, since Clapper is not concerned with providing flexible tethers that do not bind to the cells of interest but rather uses positively charged molecules that encourage cell adhesion, one of skill in the art in optimizing the length of the positively charged molecules of Clapper would not have the same goals as one reading Appellants' specification. Appellants' goal was to provide flexible tethers that would allow movement of the growth effector molecules, not promote cell adhesion to the tethers. Therefore, Clapper does not suggest the claimed compositions and methods and Clapper does not indicate that the invention would have a reasonable likelihood of success.

iii. EP '733 Does Not Render The Claims Obvious

As discussed above, EP '733 does not disclose a composition or method for stimulating cell growth wherein the rate of target cell growth is enhanced. EP '733 does not suggest attaching growth effector molecules to a tether attached to a substrate at a concentration effective to enhance the rate of cell growth.

iv. WO '616 Does Not Render The Claims Obvious

As discussed above, WO '616 does not describe or suggest how to tether growth effector molecules to a substrate so as to enhance the rate of growth of cells over the rate of cell growth with soluble or adsorbed growth effector molecules.

v. The Combination of Herweck And Merrill '264 Does Not Render The Claims Obvious

Herweck discloses implantable devices for sustained release of a bioactive material, such as a therapeutic agent, a cell type, or a diagnostic agent, into a fluid flow pathway of a patient (see column 3, lines 14-16 and 30-37). Herweck discloses first coating or modifying the surface with glycoproteins such as fibronectin prior to seeding the device with cells (see column 4, lines 62-68). Herweck discloses that such coating may result in improved adhesion of cells (see column 6, lines 23-29). As recognized by the Examiner, Herweck does not disclose or suggest the use of a tether attaching a growth effector molecule to a substrate but merely coats, or adsorbs, the factor upon the substrate.

Merrill '264 discloses star molecules of polyethyleneoxide (PEO) that are biocompatible and demonstrate non-thrombogenic properties. Merrill '264 (and Merrill, J. Biomater. Sci. Polymer. 5, 1-11 (1993) ("Merrill") also cited by the Examiner) disclose the type of star molecules that are useful in Appellants' compositions and methods, as discussed in the specification at page 7, lines 3-20.

There is no suggestion in either reference to incorporate the teaching of the other reference. Herweck does not suggest that it would be advantageous to tether the factors to the substrate. Merrill '264 does not suggest using the star molecules for tethering growth effector molecules to a substrate. The primary use for the star molecules that is described in

Merrill '264 is for separating and purifying therapeutic proteins. Other proposed uses are described at column 6, lines 6-27.

Moreover, even if the teachings of the references are combined, the combination does not suggest the claimed compositions or methods because it does not suggest attaching growth effector molecules in a concentration and with tethers to a substrate so that cell growth is enhanced. Neither Merrill reference suggests how to tether growth effector molecules to alter cell growth. Even if one of skill in the art used PEO tethers in the device taught by Herweck in order to prevent thrombogenesis, as suggested by the Examiner, there is no teaching in the references on how to do so to enhance cell growth.

There is no teaching in either reference to select bioactive molecules enhancing cell growth rate in the amount required to enhance growth rate when not internalized and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

In fact, Merrill '264 teaches away from the claimed compositions and methods because it discloses that the PEO star molecules are non-thrombogenic, i.e., do not absorb proteins of the intrinsic clotting system or of the platelet membrane (see Merrill, column 1, lines 6-9). One of ordinary skill in the art would thus know that the use of PEO as a tether would tend to repel cells, and would thus believe that PEO would not allow contact of the attached growth effector molecules with the cells.

Appellants use PEO, or similar, polymeric tethers, which are water soluble so that they unfold in solution and provide flexibility to the molecules tethered thereon, and yet in Appellants' compositions and methods the growth effector molecules are able to contact cells so as to provide enhanced cell growth.

vi. The Combination of Herweck, Merrill '264 And Merrill Does Not Render The Claims Obvious

Merrill is cited as teaching PEO having a length between 136 and 1360 atoms. However, the newly cited reference does not correct the deficiencies of the other references, Herweck and Merrill '264, as discussed above. Merrill also does not suggest tethering growth effector molecules to a substrate in an amount effective to enhance the rate of cell growth.

vii. The Combination Of Herweck, Merrill '264 And Mikos Does Not Render The Claims Obvious

Claim 17 is dependent upon claim 13 and intervening claims 14-16, which, as discussed above, are not taught or made obvious by Herweck and Merrill '264. Mikos does not add the elements missing from the Herweck/ Merrill combination.

Mikos is similar to Herweck in that it is directed to a matrix for seeding with cells that can be implanted. It also does not disclose or make obvious selecting bioactive molecules enhancing growth rate, determining the amount required to enhance growth rate

when not internalized, or chemically coupling the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

viii. The Combination Of Herweck, Merrill '264 And Naughton Does Not Render The Claims Obvious

Naughton is actually quite similar to Clapper. It discloses a matrix which might be suitable for implantation, having attached thereto stromal cells that serve as attachment factors for other types of cells grown on the matrix. One skilled in the art would be led by the disclosure of Naughton to believe that no further modifications were necessary in order to grow cells since the stromal cells result in adequate cell attachment and growth. In fact, this material is in clinical use and works quite well as a skin substitute.

ix. The Combination Of Herweck, Merrill '264 And Tomomura Does Not Render The Claims Obvious

Tomomura also does not contain a suggestion for combining the individual teachings of Herweck and Merrill '264 and does not disclose or make obvious selecting bioactive molecules enhancing growth rate, determining the amount required to enhance growth rate when not internalized, and chemically coupling the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

In summary, none of the art provides the critical recognition that one can further enhance the effect of known growth effector molecules on the rate of cell growth by tethering the molecules to a substrate, particularly using flexible and branched tethers.

e. Drawing Rejection

The drawings were objected to for using the term "coupled" rather than "tethered" as is used in the specification. A proposed drawing change showing the change in red ink was submitted with the Amendment mailed July 14. In the Examiner Summary Interview Record of the Interview conducted August 25, 1997, the Examiner indicates that the drawing change was received and approved.

(9) SUMMARY

The cited prior art references do not teach or suggest compositions or methods for enhancing cell growth involving the use of a water soluble polymeric tether attached to a substrate that is able to bind more than one growth effector molecule so that the molecules cannot be internalized by the cell and the growth of target cells is enhanced as compared to the rate of cell growth of cells exposed to soluble and adsorbed growth effector molecules.

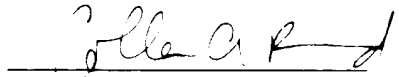
Indeed, it is surprising that Applicants obtained the results observed, because there was as great a likelihood that the coupled growth factors would sterically hinder binding of the growth factors to the cell, actually decreasing the effectiveness of the growth factors on

the cell growth rate, as there was that the same, much less enhanced, rate of growth would be observed when growth factors were administered in soluble form or coupled singly to the substrate. Nowhere has the Examiner pointed to any literature that would indicate that one skilled in the art would predict that the claimed compositions would be effective to enhance cell growth rate in any manner differently, much less better than, the same growth effector molecule immobilized on the substrate.

(10) CONCLUSION

For the foregoing reasons, Appellants submit that claims 1-32 are novel and non-obvious over the prior art and their allowance is earnestly solicited.

Respectfully submitted,



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CERTIFICATE OF MAILING UNDER 37 CFR §1.8a

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.

Date: November 24, 1997

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Collen A. Beard

APPENDIX I

Claims as Pending in the Application

1. (once amended) A composition for stimulating the growth of eukaryotic cells comprising

a biocompatible solid substrate,

biocompatible synthetic polymeric tethers, and

growth effector molecules,

wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules.

2. The composition of claim 1 wherein the form of the biocompatible substrate is selected from the group consisting of netting, individual and woven fibers, sponge and shaped polymers.

3. The composition of claim 2 wherein the shape of the shaped polymer is selected from the group consisting of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape.

4. The composition of claim 1 wherein the biocompatible substrate is selected from the group consisting of glasses, metals and biocompatible polymers.

5. (once amended) The composition of claim 4 wherein the polymer is selected from the group consisting of synthetic polymers and natural polymers.

6. (once amended) The composition of claim 5 wherein the polymer is selected from the group consisting of proteins, polysaccharides, extracellular matrix proteins, polyesters, polycapralactone, polyhydroxybutyrate, polyanhydrides, polyphosphazenes, polyorthoesters, polyurethanes, and combinations thereof.

7. The composition of claim 1 wherein the tether is a water soluble, biocompatible polymer.

8. The composition of claim 7 wherein the tether is selected from the group consisting of polyethylene oxide, carboxymethylcellulose, and starch.

9. (once amended) The composition of claim 1 wherein the growth effector molecules are selected from the group consisting of epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, and combinations thereof.

10. The composition of claim 1 wherein the tether has a backbone length between 5 and 50,000 atoms.

11. The composition of claim 10 wherein the tether has a backbone length between 100 and 50,000 atoms.

12. The composition of claim 10 wherein the tether has a backbone length between 5 and 500 atoms.

13. (once amended) A method for growing eukaryotic cells comprising
bringing into contact the cells and a composition comprising
a biocompatible solid substrate,
biocompatible polymeric tethers, and
growth effector molecules,
wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules; and
maintaining the contacting cells and composition under conditions and for a time sufficient to cause the cells to grow.

14. The method of claim 13 wherein the step of bringing into contact comprises administering the composition to a patient in need of cell growth.

15. The method of claim 14 wherein the composition is administered by injection, infusion, or implantation.

16. The method of claim 15 wherein the composition is administered by implantation of the composition and wherein the substrate is shaped to match a desired tissue shape.

17. The method of claim 16 wherein the substrate is biodegradable.

18. The method of claim 13 wherein the form of the biocompatible substrate is selected from the group consisting of netting, individual and woven fibers, sponges and shaped polymers.

19. The method of claim 18 wherein the shape of the shaped polymer is selected from the group consisting of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape.

20. The method of claim 13 wherein the biocompatible substrate is selected from the group consisting of glasses and biocompatible polymers.

21. The method of claim 20 wherein the polymer is selected from the group consisting of synthetic polymers and natural polymers.

22. The method of claim 21 wherein the polymer is selected from the group consisting of polylactic acid, polyglycolic acid, polyanhydrides, polyorthoesters, collagen, glycosaminoglycans, polyamino acids, and combinations thereof.

23. The method of claim 13 wherein the tether is a water soluble, biocompatible polymer.

24. The method of claim 23 wherein the tether is selected from the group consisting of polyethylene oxide, carboxymethylcellulose, and starch.

25. (once amended) The method of claim 13 wherein the growth effector molecules are selected from the group consisting of epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, and combinations thereof.

26. The method of claim 13 wherein the tether has a backbone length between 5 and 50,000 atoms.

27. The method of claim 26 wherein the tether has a backbone length between 100 and 50,000 atoms.

28. The method of claim 13 wherein the tether has a backbone length between 5 and 500 atoms.

29. (once amended) The method of claim 13 wherein the cells are selected from the group consisting of parenchymal cells and stem cells.

30. The method of claim 29 wherein the cells are hepatocytes.

31. (once amended) A cell culture comprising
a biocompatible solid substrate,
biocompatible polymeric tethers,
growth effector molecules, and
growing cells,
wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules, and wherein the growing cells are bound to the growth effector molecules.

32. (once amended) A method of testing a compound for an effect on tissue comprising
bringing into contact the compound to be tested and a composition comprising
a biocompatible solid substrate,
biocompatible polymeric tethers,
growth effector molecules, and
growing cells,
wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth without internalization of the molecules, and wherein the growing cells are bound to the growth effector molecules;
incubating the compound and the composition under conditions promoting cell growth; and
observing the cells for any effect not observed in cells not brought into contact with the composition.

APPENDIX II

Claims As They Would Be Pending upon Entry of the Amendment Hand Delivered September 4, 1997
With additions underlined and deletions bracketed.

1. (twice amended) A composition for stimulating the growth of eukaryotic cells comprising

a biocompatible solid substrate,

biocompatible synthetic <u>branched water soluble</u> polymeric tethers, and

growth effector molecules,

wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, <u>each tether is able to covalently link more than one growth effector molecule,</u> and

the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth <u>over the rate of target cell growth with soluble growth effector molecules and growth effector molecules adsorbed to a substrate</u> without internalization of the molecules.

2. The composition of claim 1 wherein the form of the biocompatible substrate is selected from the group consisting of netting, individual and woven fibers, sponge and shaped polymers.

3. The composition of claim 2 wherein the shape of the shaped polymer is selected from the group consisting of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape.

4. The composition of claim 1 wherein the biocompatible substrate is selected from the group consisting of glasses, metals and biocompatible polymers.

5. (twice amended) The composition of claim 4 wherein the <u>substrate</u> polymer is selected from the group consisting of synthetic polymers and natural polymers.

6. (twice amended) The composition of claim 5 wherein the <u>substrate</u> polymer is selected from the group consisting of proteins, polysaccharides, [extracellular matrix proteins,]polyesters, polycapralactone, polyhydroxybutyrate, polyanhydrides, polyphosphazenes, polyorthoesters, polyurethanes, and combinations thereof.

7. (cancelled)

8. (amended) The composition of claim 1 [7] wherein the tether is selected from the group consisting of polyethylene oxide[,] and carboxymethylcellulose[, and starch].

9. The composition of claim 1 wherein the growth effector molecules are selected from the group consisting of epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, and combinations thereof.

10. The composition of claim 1 wherein the tether has a backbone length between 5 and 50,000 atoms.

11. The composition of claim 10 wherein the tether has a backbone length between 100 and 50,000 atoms.

12. The composition of claim 10 wherein the tether has a backbone length between 5 and 500 atoms.

13. (twice amended) A method for growing eukaryotic cells comprising

(a) bringing into contact the cells and a composition comprising

a biocompatible solid substrate,

biocompatible branched water soluble polymeric tethers, and

growth effector molecules,

wherein one end of each tether is covalently linked to the substrate, each tether is able to covalently link more than one growth effector molecule, [and]

each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, and

the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth over the rate of target cell growth with soluble growth effector molecules and growth effector molecules adsorbed to a substrate, without internalization of the molecules; and

(b) maintaining the contacting cells and composition under conditions and for a time sufficient to cause the cells to grow.

14. The method of claim 13 wherein the step of bringing into contact comprises administering the composition to a patient in need of cell growth.

15. The method of claim 14 wherein the composition is administered by injection, infusion, or implantation.

16. The method of claim 15 wherein the composition is administered by implantation of the composition and wherein the substrate is shaped to match a desired tissue shape.

17. The method of claim 16 wherein the substrate is biodegradable.

18. The method of claim 13 wherein the form of the biocompatible substrate is selected from the group consisting of netting, individual and woven fibers, sponges and shaped polymers.

19. The method of claim 18 wherein the shape of the shaped polymer is selected from the group consisting of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape.

20. The method of claim 13 wherein the biocompatible substrate is selected from the group consisting of glasses and biocompatible polymers.

21. (amended) The method of claim 20 wherein the substrate polymer is selected from the group consisting of synthetic polymers and natural polymers.

22. (amended) The method of claim 21 wherein the substrate polymer is selected from the group consisting of polylactic acid, polyglycolic acid, polyanhydrides, polyorthoesters, collagen, glycosaminoglycans, polyamino acids, and combinations thereof.

23. (cancelled)

24. (amended) The method of claim 13 [23] wherein the tether is selected from the group consisting of polyethylene oxide, carboxymethylcellulose, and starch.

25. The method of claim 13 wherein the growth effector molecules are selected from the group consisting of epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone

morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, and combinations thereof.

26. The method of claim 13 wherein the tether has a backbone length between 5 and 50,000 atoms.

27. The method of claim 26 wherein the tether has a backbone length between 100 and 50,000 atoms.

28. The method of claim 13 wherein the tether has a backbone length between 5 and 500 atoms.

29. The method of claim 13 wherein the cells are selected from the group consisting of parenchymal cells and stem cells.

30. The method of claim 29 wherein the cells are hepatocytes.

31. (twice amended) A cell culture comprising
a biocompatible solid substrate,
biocompatible branched water soluble polymeric tethers,
growth effector molecules, and
growing cells,
wherein one end of each tether is covalently linked to the substrate, each tether is able to covalently link more than one growth effector molecule, [and]
each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, [and] the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth over the rate of target cell growth with soluble growth effector molecules and growth effector molecules adsorbed to a substrate, without internalization of the molecules, and wherein the growing cells are bound to the growth effector molecules.

32. (twice amended) A method of testing a compound for an effect on tissue comprising
(a) bringing into contact the compound to be tested and a composition comprising
a biocompatible solid substrate,
biocompatible branched water soluble polymeric tethers,
growth effector molecules, and
growing cells,

wherein one end of each tether is covalently linked to the substrate, each tether is able to covalently link more than one growth effector molecule, [and]

each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, [and]

the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth over the rate of target cell growth with soluble growth effector molecules and growth effector molecules adsorbed to a substrate, without internalization of the molecules, and wherein the growing cells are bound to the growth effector molecules;

(b) incubating the compound and the composition under conditions promoting cell growth; and

(c) observing the cells for any effect not observed in cells not brought into contact with the composition.

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Appendix II: Claims As They Would Be Pending upon Entry of the Amendment Hand Delivered September 4, 1997

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